MOLECULAR ANALYSIS OF SOLIDASTER CV. LEMORE, A HYBRID GOLDENROD (ASTERACEAE)

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ABSTRACT

Analysis of nuclear ribosomal ITS and ETS sequence data was used to assess the relationships of the garden ornamental Solidaster cv. Lemore, a cultivar that was at one time believed to be an intergeneric hybrid (*Solidago* × *Aster*). Part of the analysis involved assessment of the generic placement of one of its putative parents, the Upland White Aster, *Solidago ptarmicoides*. Because of its superficial external appearance this species is still often treated as an *Aster*, although there is evidence from several sources that suggests it should be classified within *Solidago*. Phylogenetic analysis of the combined ITS and ETS sequence data showed that there was strong support for the inclusion of *S. ptarmicoides* within *Solidago*, based on its placement in a well supported clade composed of all other sampled species of the genus. The ITS sequence data of Solidaster showed evidence of a hybrid origin based on the presence of several intraindividual base pair polymorphisms. Cloning experiments recovered two different individual ITS sequences, one identical to that obtained from *S. ptarmicoides* and a second that matched sequences obtained for *S. canadensis*. Thus DNA sequence data suggested that Solidaster cv. Lemore is a hybrid goldenrod that involved a cross between *S. ptarmicoides* and *S. canadensis*. The data further indicated that another possible candidate, *Euthamia graminifolia*, is not a parent of Solidaster. Also notable was the striking lack of sequence divergence for ITS and ETS among species of *Solidago*, suggesting that estimation of phylogenetic relationships within the genus will require more rapidly evolving markers.

KEY WORDS: Asteraceae, Astereae, Solidaster, *Solidago*, ITS, ETS, hybrid, phylogeny

RESUMEN

Se realizó un análisis con los datos de las secuencias del ITS y ETS nuclear ribosómico para evaluar las relaciones de la planta ornamental Solidaster cv. Lemore, un cultivar que durante un tiempo se creyó que era un híbrido intergenérico (*Solidago* × *Aster*). Parte del análisis implicó la evaluación de la colocación del género de uno de los padres putativos, *Solidago ptarmicoides*. Debido al parecido externo de esta especie esta especie se trata a menudo como un *Aster*, aunque hay pruebas de varias fuentes que sugieren que debería clasificarse en *Solidago*. El análisis filogenético de datos combinado de las secuencias de los ITS y ETS mostró que hay un respaldo fuerte para la inclusión de *S. ptarmicoides* en *Solidago*, basado en su colocación en un clado bien respaldado compuesto por todas las otras especies muestreadas del género. Los datos de la secuencia del ITS de Solidaster mostraron pruebas de un origen híbrido basado en la presencia de varios polimorfismos de pares intraindividuales. Los experimentos de clonación recogieron dos secuencias de ITS individuales diferentes, una idéntica a la obtenida a partir de *S. ptarmicoides* y la segunda que coincidía con secuencias obtenidas de *S. canadensis*. Por tanto los datos de la secuencia de DNA sugieren que Solidaster cv. Lemore es una vara de oro híbrida procedente de un cruce entre *S. ptarmicoides* y *S. canadensis*. Los datos indicaron además que otro candidato posible, *Euthamia graminifolia*, no es un parental de Solidaster. Fue también notable la falta de divergencia en la secuencia de ITS y ETS entre especies de *Solidago*, sugiriendo que la estimación de relaciones filogenéticas requerirá marcadores de evolución más rápida.

INTRODUCTION

Consideration of the role of hybridization in evolution has received new impetus with the availability of molecular genetic data (Arnold 2006). It has long been clear that interspecific hybridization is a widespread

phenomenon in plants, with numerous clearly documented examples (e.g., Grant 1980), but there are still questions regarding its evolutionary significance. Of perhaps greatest interest is hybridization between species of lineages that have diverged morphologically to the point of being recognized as distinct genera. This represents a potentially dramatic reversal of the process of evolution, which is normally considered to be divergent, and it also presents a challenge to systematic classification based on phylogeny. It is thus of considerable interest to examine closely possible cases of intergeneric hybridization to be able to assess how frequently this phenomenon occurs.

A persistent myth is that intergeneric hybridization is common and perhaps even pervasive within Asteraceae. For example, Robinson (1983) and King and Robinson (1987) invoke intergeneric hybridization as an explanation for discordant distribution of morphological characters in Liabeae and Eupatorieae, respectively. A search of the literature, however, reveals few well documented cases of natural intergeneric hybridization in Asteraceae. Naturally occurring intergeneric hybrids have recently been documented by McKenzie et al. (2004) and Saito et al. (2006). In some cases, artificial intergeneric hybrids have been made successfully (e.g., Powell 1985; Carr 2003), and studies have found incongruence between nuclear and chloroplast markers suggestive of past wide hybridization (Schilling & Panero 1996; Fehrer et al. 2007). But documentation of intergeneric hybrids in most groups remains elusive. This prompted us to undertake a detailed study of a putative case of intergeneric hybridization within the Astereae.

The name of the horticultural plant known as Solidaster cv. Lemore (Fig. 1) reflects its presumed hybrid origin as a cross between species of *Solidago* L. and *Aster* L. The plant is unknown in the wild, but appeared in European gardens after the import of plants from North America (Nesom 1993). Its status as an intergeneric hybrid depends in part, however, on the classification of one of its putative parents, the Upland White Aster, which has been variously placed in *Aster*, *Solidago*, and *Oligoneuron* Small. The Upland White Aster, *S. ptarmicoides* (Torrey & A. Gray) B. Boivin, has long been a source of puzzlement for taxonomists. Its superficial outward appearance, especially its open corymbose capitulescence and relatively large heads with white rays and disk flowers, seems clearly that of an aster, as traditionally conceived, and is reflected in its common name. Despite this, considerable evidence has suggested a closer affinity to the goldenrods, including the observation of abundant natural hybrids with species of *Solidago* (Boivin 1972), considerations of phytogeography and technical features of morphology (Brouillet & Semple 1981), and a chloroplast DNA restriction site study placing it within *Solidago* L. (Semple et al. 1999). Nevertheless, it is still widely referred to as an *Aster* s.l. Another, somewhat different interpretation involves segregation of the genus *Oligoneuron* as distinct from *Solidago* (e.g., Nesom 1993), which would include the Upland White Aster as well as five other species. Although the second parent of Solidaster has generally been considered to be a *Solidago*, particularly *S. canadensis* (Brouillet & Semple 1981), Nesom (1993) suggested that based on morphology it was more likely to be *Euthamia graminifolia* (L.) Nutt.

The taxonomic history of the Upland White Aster, *Solidago ptarmicoides*, has been reviewed by Brouillet and Semple (1981), and only a brief summary will be presented here. Not only the generic placement but also its species epithet has varied. The plant was originally described by Nuttall in *Inula* L., where it received the appropriate epithet *alba* for its white rays and disk flowers. When transferred to either *Aster* or *Solidago* this epithet proved to be already occupied, and the alternative *ptarmicoides* (from its resemblance to *Achillea ptarmica* L., a widely cultivated garden plant; Fernald 1950) was proposed by Nees. When placed in still another genus, such as *Oligoneuron* (e.g., Nesom 1993), it reverts to the epithet *album*. Over its taxonomic history, the species has been placed in nine separate genera by various authors (Semple & Cook 2006).

The taxonomic treatment of *Solidago* has been notoriously difficult (Semple & Cook 2006). Although there are a large number of relatively similar species in eastern North America, no clearcut morphological apomorphy has been discovered that unequivocally defines the genus. Similarly, delimitation of infrageneric groups has been elusive as has recognition and identification of individual species, many of which differ from one another by minute details of inflorescence form or pubescence. The situation is further complicated by the frequent occurrence of both interspecific hybridization and polyploidy. Some representatives

Fig. 1. Solidaster cv. Lemore, habit and close up of inflorescence.

of the genus are quite common and widespread and are particularly significant as dominants during early successional stages of old fields, where several are components of a well studied ecological model system (e.g., Abrahamson et al. 2005). Additionally, *Solidago* taxa have become invasive in Europe (Weber 2001) and Asia (Dong et al. 2006), and are being used as models to understand the general evolution and ecology of invasive species (Van Kleunen & Schmidt 2003; Jakobs et al. 2004; Meyer et al. 2005). The abundance and ecological importance of *Solidago* therefore make a sound taxonomy highly desirable.

A number of studies have applied molecular data to the resolution of systematic problems in Astereae (Suh & Simpson 1990; Morgan & Simpson 1992; Lane et al. 1996; Zhang 1996; Noyes & Rieseberg 1999; Roberts & Urbatsch 2003; Urbatsch et al. 2003; Beck et al. 2004), so a considerable body of molecular data has begun to accumulate for the tribe. Other than a chloroplast DNA restriction site analysis (Zhang 1996; Semple et al. 1999), there has been no focus as yet for DNA sequence analysis on *Solidago*, although a few sequences are available from other work. As part of an initial survey for suitable molecular markers, we analyzed ITS and ETS sequence variation for several samples of *Solidago* that represented the major subunits of the genus, as delimited by Semple and Cook (2006). Although the lack of suitable variation in these markers led us to abandon any attempt to make a complete survey of the genus, one of the species that we sampled was *S. ptarmicoides*, and sufficient data have been acquired to allow for an assessment of its generic placement, as well as to clarify the parentage of its hybrid offspring, Solidaster cv. Lemore, which we report here.

MATERIALS AND METHODS

Plant Material

A sample of Solidaster cv. Lemore (Fig. 1) was grown from material obtained commercially (Bluestone Perennials, Ohio, USA). Samples of *Solidago* (Appendix) were either collected in the field or obtained from herbarium specimens. Sampling was designed to include at least one member of each of the currently recognized sections and subsections of the genus (Semple & Cook 2006). To evaluate intraspecific variability, multiple accessions of four species (*S. canadensis*, *S. riddellii*, *S. ptarmicoides*, and *S. nitida)* as well as an additional sample of *Brintonia discoidea* were analyzed. Note that we have elected to follow the most recent treatment of *Solidago* (Semple & Cook 2006), which deviates from nomenclature used in our earlier paper

(Beck et al. 2004) by including *Oligoneuron* within *Solidago* but recognizing *Brintonia* as separate from it; the classification of related genera also follows the Flora of North America treatments.

Molecular Methods

Preparations of total DNA made from fresh (0.5–2.0 g) leaves generally followed the procedure of Doyle and Doyle (1987), and those from herbarium material (ca. 0.1 g) were performed with the Dneasy Plant Minikit (Qiagen, Valencia CA). The crude DNA extracts of some samples required further purification using the Wizard Kit protocol (Promega, Madison, Wisconsin). Methods for DNA amplification and sequencing were as described in Schmidt and Schilling (2000). Amplification and sequencing reactions for the ITS region were both performed using primers "ITS-4" and "ITS-5" (White et al. 1990). Amplification and sequencing reactions for the ETS region were performed using primers Ast-1 (Markos & Baldwin 2001) and 18-S-ETS (Baldwin & Markos 1998). All PCR products were checked by agarose gel electrophoresis. Sequencing was done at the University of Tennessee Automated Sequencing Facility, utilizing the ABI Prism Dye Terminator Cycle Sequencing reaction kit on an ABI 373 or ABI 3100 DNA sequencer (Perkin-Elmer Inc. Foster City, CA). The initial sequence data text files were edited following comparison with the same data displayed in four-color electropherograms before further analysis. Sequence alignment was performed using the Clustal X (version 1.6) program (Thompson et al. 1994).

Cloning of the ITS region for the sample of Solidaster cv. Lemore was undertaken to confirm ITS polymorphisms inferred from direct sequencing. The purified PCR products were ligated into pGEM-T (Promega, Madison, Wisconsin) according to the manufacturer's instructions. Competent Top10 F' (Invitrogen, San Diego, California) cells were transformed via electroporation and the resulting colonies were screened for plasmids with inserts by PCR using the original amplification primers. Plasmids were isolated from single recombinant colonies using an alkaline lysis/PEG precipitation protocol (Sambrook et al. 1989). Sequences were obtained for ten independent clones.

Data for additional ITS and ETS sequences for *Solidago* were obtained from GenBank, as well as for all of the samples placed in the same clade as *Solidago* s.s. in Beck et al. (2004; "Clade III"). Two data sets were analyzed, the first of which utilized ITS sequences from a broad sampling of *Solidago*, with samples of *Brintonia discoidea* as outgroups. The second utilized both ITS and ETS data with a broader sampling of related genera (Appendix) to assess the placement of *Solidago ptarmicoides* and of *Oligoneuron* relative to other *Solidago*, and samples of *Sericocarpus tortifolius* and *Cuniculotinus gramineus* were added as outgroups for this analysis. Phylogenetic relationships were analyzed using both maximum parsimony and Bayesian approaches. Parsimony analysis was implemented using PAUP* 4.0b10 (Swofford 2003), with gaps treated as missing data, using a heuristic search with 1,000 random addition replicates and with TBR branch swapping. Bootstrap analysis (Felsenstein 1985) was performed with 10,000 replicates using the FASTSTEP search option. Bayesian analysis was implemented in MRBAYES 3.0B4 (Huelsenbeck & Ronquist 2001) run for ten million generations with four separate chains and trees saved every 100 generations. The number of trees to discard as "burn-in" was assessed by plotting likelihoods of trees sampled throughout the run and discarding all trees prior to the stable likelihood plateau (in both analyses the first 10% were discarded). An appropriate maximum likelihood model of sequence evolution (GTR+I+G; General Time Reversible model with a proportion of invariant sites and gamma distributed rates) for the Bayesian analysis was chosen for both analyses using Modeltest (Posada & Crandall 1998).

RESULTS

Newly obtained ITS sequences for Solidaster cv. Lemore and for *Solidago* were consistent in length with previous reports for the genus. In particular, there was extraordinarily little variation in length among any of the sampled members of the genus; individual sequence lengths varied from 627–629 bp. Insertion of single 1 bp indels were required for five samples (three insertions and two deletions) to produce a completely aligned matrix of length 631 bp for the entire ITS region. Similarly, there was little sequence length variation in allied genera, with individual sequences varying from 627–630 bp, and the aligned matrix was of

length 634. There was also only limited sequence variation between species of *Solidago* for the ITS region; pairwise comparisons revealed sequence divergence of generally less than 1%, with total differences between samples of 1–10 bp. Samples of other genera were generally, although not always, more divergent, with pairwise divergence values of 1–3% and total differences of 7–19 bp. The sample of *Sericocarpus tortifolius* differed from all *Solidago* samples for at least 15 bp, but was still relatively similar, with overall divergence values of 2–3%; the sample of *Cuniculotinus gramineus* was somewhat more divergent, differing by 23–29 bp (4% overall divergence) from samples of *Solidago*. In the five species (*S. canadensis*, *S. riddellii*, *S. nitida*, *S. ptarmicoides*, and *Brintonia discoidea*) where multiple accessions were examined there was at most one bp difference among samples and no evidence of nonmonophyly.

The ITS sequence for Solidaster cv. Lemore was very similar to other *Solidago* sequences, but displayed bp polymorphisms (Fig. 2) at the following positions: 18 (A/G), 106 (C/A); 514 (C/T), 611 (G/T), 612 (C/T), 613 (A/G). Visual inspection revealed that this pattern of bp polymorphisms would fit exactly with a combination of the ITS sequences characteristic of *S. ptarmicoides* and *S. canadensis* (Fig 2.) Cloned ITS repeats from Solidaster cv. Lemore exhibited ITS sequences that matched closely either those of *S. ptarmicoides* or those of *S. canadensis*. Phylogenetic analysis in comparison to a broad sampling of *Solidago* showed that consensus sequences from the clones clustered with some statistical support with those from *S. ptarmicoides* and *S. canadensis* (Fig. 3), which provided supporting evidence that the polymorphisms obtained from direct sequencing could have originated from a combination of the sequences from these species. The ITS sequence of *Euthamia graminifolia*, proposed by Nesom (1993) as the second potential parent of Solidaster, has been shown to be relatively divergent from those of *Solidago* (Noyes and Rieseberg 1999), and its reported ITS sequence (GenBank AF046982) differs from that of *S. ptarmicoides* by 4 indels as well as a minimum of 32 bp. Because the ITS sequence of Solidaster cv. Lemore did not show any evidence of indel polymorphisms, and only a few bp polymorphisms, *Euthamia graminifolia* is clearly not one of its parents.

Approximately 450 bp of the ETS region were amplified for each sample of *Solidago* with the primer pair 18S-ETS and Ast-1. Removing regions of poor sequence and uncertain alignment produced a matrix with a total length of 444 bp, including 18 bp of 18S coding sequence. As with ITS, length variation among sequences was minimal among *Solidago* samples: only a single 1 bp deletion in *S. patula*, and only a single 1 bp insertion in *Stenotus* and a 3 bp deletion in both outgroup taxa, *Cuniculotinus* and *Sericocarpus*, were required for complete alignment of this portion of the ETS region. Levels of sequence divergence for ETS were similar to those for ITS, with pairwise divergence values among *Solidago* samples of 0–1% (0–4 bp) and among all samples reaching a maximum of 4% (17 bp). The ETS sequence of Solidaster cv. Lemore was basically identical to that of *S. ptarmicoides*, although each sequence exhibited a single, non-informative bp polymorphism (position 124 in Solidaster, 36 in *S. ptarmicoides*; both A/G). Other than the polymorphic positions, the ETS sequence of *S. canadensis* differed from those of *S. ptarmicoides* at only a single position, and there was no evidence of polymorphism in the sequence of Solidaster cv. Lemore at this position.

For phylogenetic analysis to assess the placement of *S. ptarmicoides* relative to *Solidago*, the ITS and ETS sequence data were analyzed together, producing a combined matrix of 1078 bp in which there were 46 potentially parsimony-informative characters and 85 additional variable but parsimony-uninformative characters. The results of phylogenetic analyses (Fig. 4) reflected the overall low levels of divergence in providing a poorly resolved tree with relatively low levels of support for many branches. The results of the parsimony and Bayesian analyses were topologically consistent although the Bayesian tree was more resolved. A monophyletic group corresponding to clade III of Beck et al. (2004) was defined relative to *Cuniculotinus gramineus* and *Sericocarpus tortifolius* with a posterior probability of 1.00 (Bayesian) and 100% (bootstrap), respectively. At the next level of branching there was a large polytomy in the strict consensus tree, within which there was a strongly supported clade formed by the three species of *Chrysothamnus, C. scopulorum, C. stylosus*, and *C. viscidiflorus* (1.00; 99%), a variably supported clade with *Petradoria* and *Stenotus* (0.97; 57%), and a strongly supported clade formed by *Solidago*, *Chrysoma*, and *Brintonia* (1.00; 76%). Within the last clade, there was a *Chrysoma* + *Brintonia* clade (0.79; <50%) that was sister to a *Solidago* s.s. clade (1.00; 69%). Within the *Solidago* s.s. clade, there was little resolution in the consensus tree, with the only bootstrap

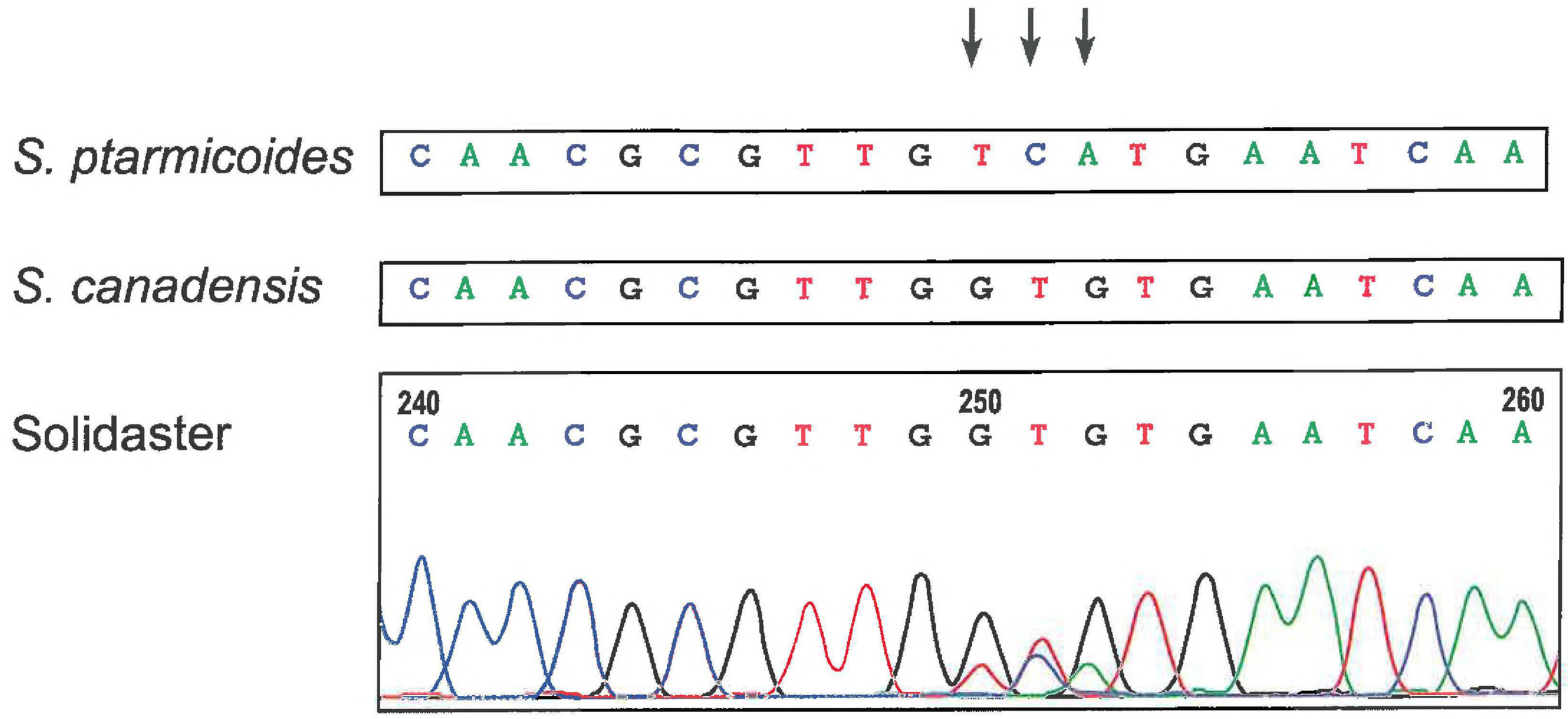

Fig. 2. Portion of an electropherogram showing base pair polymorphisms (arrows) in a portion of the ITS-2 region of Solidaster cv. Lemore compared to its putative parents, *S. canadensis* and *S. ptarmicoides*.

support greater than 80% for multispecific clades going to a strongly supported *S. canadensis* + *S. gigantea* clade (1.00; 94%); weaker support was provided to clades consisting of *S. rigida* + *S. ohiensis* (0.99; 64%), *S. caesia* + *S. riddellii* (0.62; 64%), and *S. arguta* + *S. patula* (0.50; <50%). The three samples of *S. ptarmicoides* were placed in a large polytomy with other members of the genus (Fig. 4). Results of the Bayesian analysis also provided weak to strong support for clades that received less than 50% bootstrap support, including *Petradoria/Stenotus* with *Solidago/Chrysoma/Brintonia* (0.94) and clades not present in the strict consensus tree: *Oreochrysum* + *Tonestus* (0.51), with sequential addition of *Lorandersonia* (0.52), and *Eastwoodia* (0.76), and this clade combined with the three species of *Chrysothamnus* (0.60). These results are completely congruent with those presented by Urbatsch et al. (2003) in placing *Oligoneuron* within *Solidago* as well as showing *Chrysoma* as a near outgroup to *Solidago* relative to *Sericocarpus*. They also support the revised classification of *Chrysothamnus* and related genera presented by Urbatsch et al. (2005).

DISCUSSION

Molecular data showed that Solidaster cv. Lemore is not an intergeneric hybrid by providing evidence not only to identify its parents (Figs. 2, 3) but to show clearly that both are accurately placed as members of *Solidago* (Figs. 3, 4). The combination of *Solidago ptarmicoides* and *S. canadensis* as its progenitors fits well with what is known of the origin of Solidaster, and has been proposed previously (e.g., Brouillet & Semple 1981). It has been clear based on morphology that *S. ptarmicoides* was one of the parents, and *S. canadensis* was one of the few goldenrod species naturalized near the nursery where Solidaster was first discovered (Nesom 1993). Although Solidaster does not show complete additivity in morphological features relative to *S. ptarmicoides* and *S. canadensis* (Nesom 1993), there are now numerous well documented cases of hybrids failing to exhibit one or more seemingly characteristic traits of their progenitors (Rieseberg 1995). Note that these results apply specifically to the cultivar Lemore, and other material marketed as "Solidaster" (or in florists shops as "Aster"), which may exhibit somewhat different morphologies, may not have a common origin. Laureto and Barkmann (2005, pers. comm.) present molecular-based evidence for the hybrid origin of another species, *S. houghtonii* Torr. & A. Gray ex A. Gray, that involves members of sect. *Ptarmicoidei* (*S. riddellii*) and sect. *Triplinerve* (*S. gigantea*), and similarly does not show additivity in morphology. Exclusion of *Euthamia graminifolia* as a potential parent of Solidaster also refutes another potential intergeneric hybridization hypothesis (Nesom 1993).

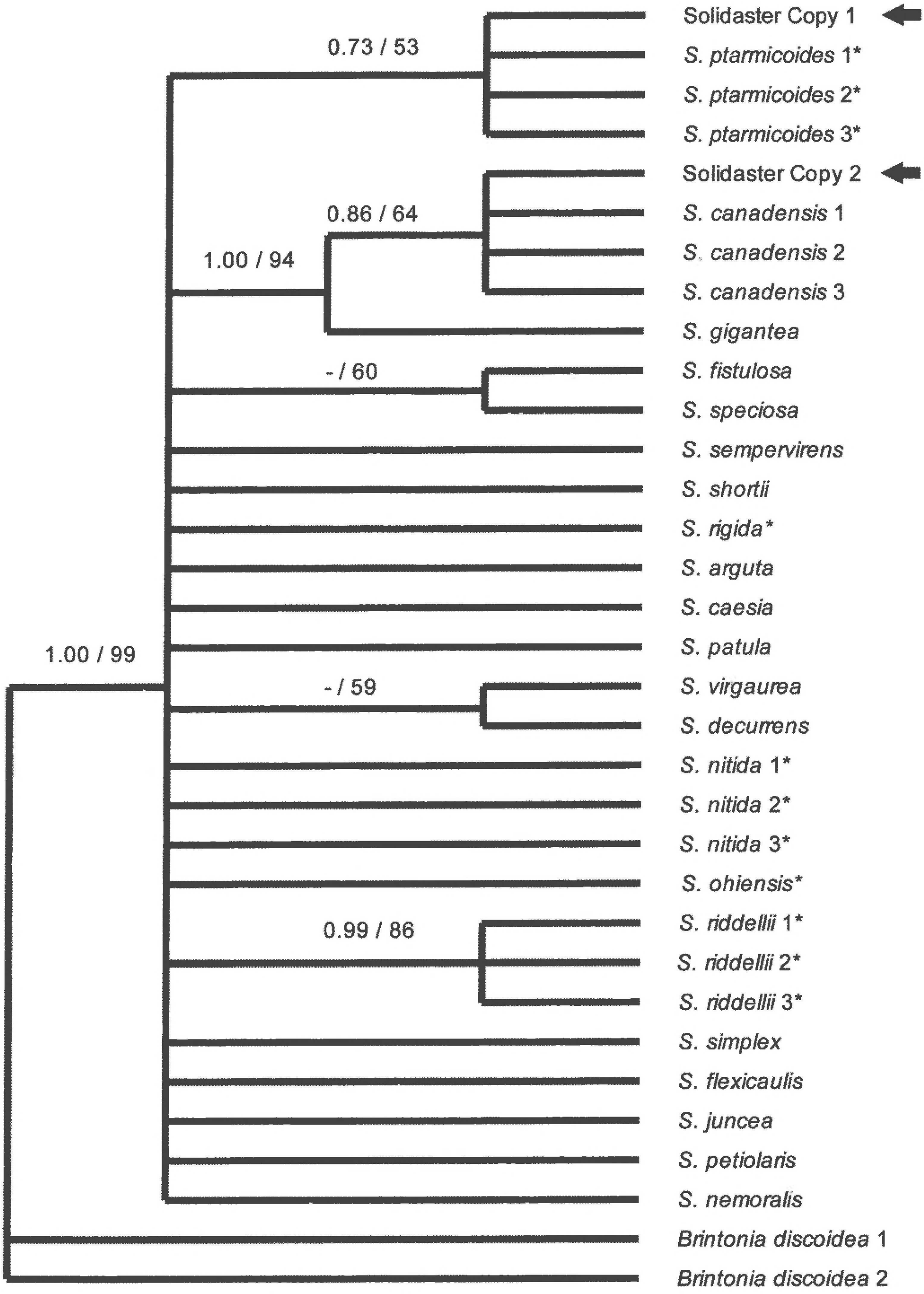

FIG. 3. Phylogenetic placement of ITS sequences from Solidaster cv. Lemore (arrows) relative to species of *Solidago* for which data were available. Shown is the strict consensus of 4576 minimum length trees in a single island (CI = 0.81 ; RI = 0.88) obtained from parsimony analysis, using *Brintonia* as outgroup. Support values from Bayesian and bootstrap (if greater than 50%) shown above branches. Asterisks designate species of *Solidago* sect. *Ptarmicoidea* (*Oligoneuron*).

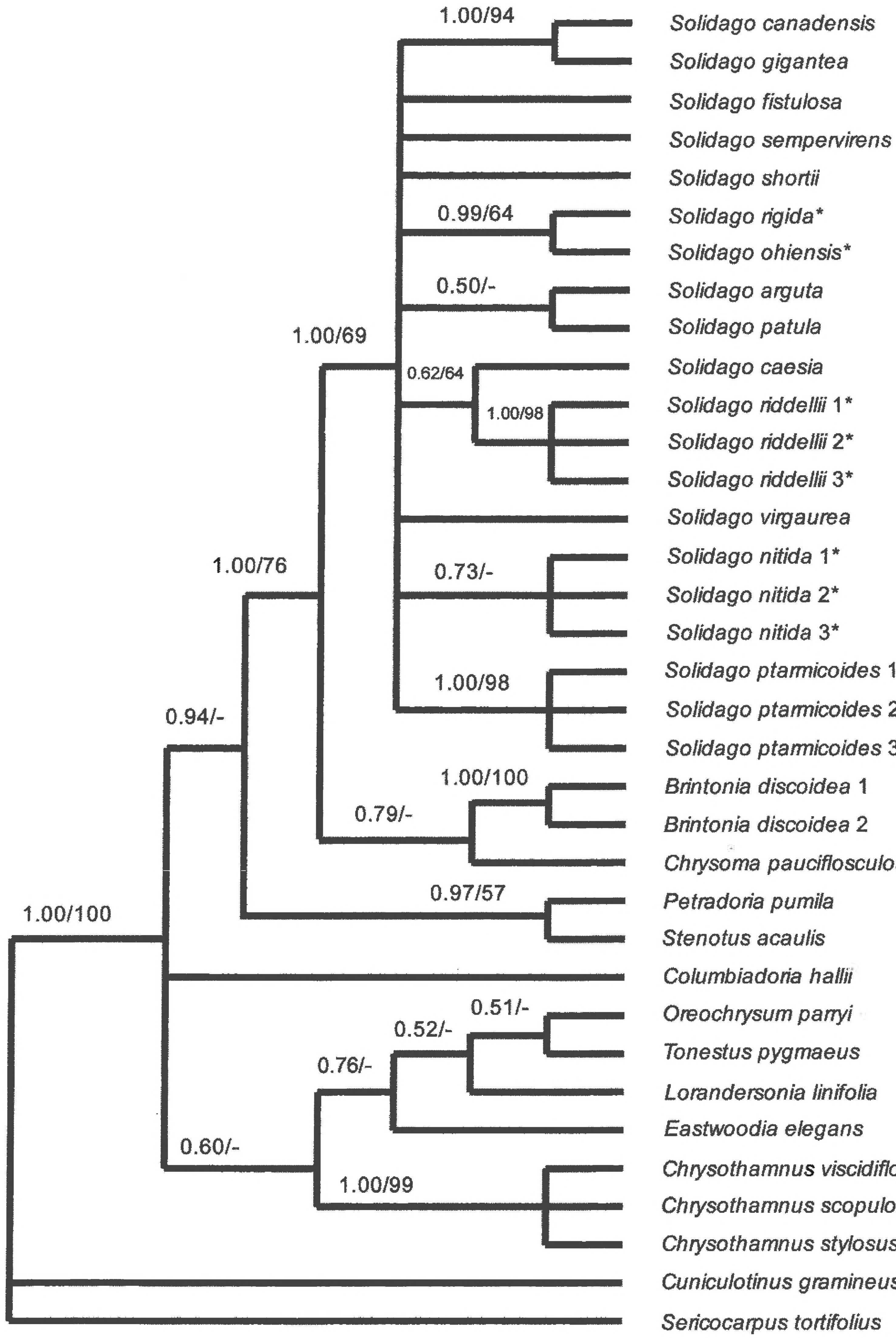

FIG. 4. Phylogenetic placement of *Solidago ptarmicoides* relative to *Solidago* and related genera, based on combined analysis of ITS and ETS data. Shown is the tree produced by Bayesian analysis (the strict consensus of 408 minimum length trees in a single island, CI=0.69, RI=0.85, obtained from parsimony analysis was topologically similar although with less resolution of clades), using *Cuniculotinus* and *Sericocarpus* as outgroups. Support values from Bayesian and bootstrap (if greater than 50%) shown above branches. Asterisks designate species of *Solidago* sect. *Ptarmicoidea* (*Oligoneuron*).

The major conclusion from phylogenetic analysis of ITS and ETS sequence data is that there is strong support for the inclusion of *S. ptarmicoides* within *Solidago*. Phylogenetic analysis clearly placed it in the clade corresponding to *Solidago* s.s (Fig. 4), and it differed in total sequence (ITS and ETS combined) from other members of *Solidago* by as little as a two bp differences (vs. *S. shortii*). There was also no support from sequence data to segregate *Oligoneuron*, represented in the data set by five of its six species including *S. ptarmicoides*, as a distinct genus; the combined ITS and ETS data in fact placed one of the *Oligoneuron* species, *S. riddellii*, with *S. caesia* from sect. *Solidago*, albeit with only weak support (Fig. 3, 4; see asterisks). The sequence data did, however, provide weak support for restricting the limits of *Solidago* to exclude *Brintonia* and *Chrysoma*, although the group formed by inclusion of these genera with *Solidago* would be monophyletic. These results largely mirror those presented by Zhang (1986; reproduced in Semple et al. 1999) based on chloroplast DNA restriction site data, and provide additional support for the most recent and comprehensive taxonomic treatment of the abovementioned taxa (Semple & Cook 2006).

A notable aspect of the ITS and ETS sequence data was the striking lack of divergence among members of *Solidago*. By contrast, ITS divergence in *Eupatorium* L. another genus of Asteraceae with a similar geographic range and occurrence in old field habitats, is 2–6% between species (12–43 bp differences; Schmidt & Schilling 2000; Schilling et al. 2007); *Eupatorium* is further differentiated from *Eutrochium* Raf., which are widely treated as congeners, by two indels (loss of a 13 bp region in *Eupatorium* and addition of a 3 bp region in *Eutrochium*) in addition to a minimum of 8% (33–61 bp) divergence. It should be noted that a previous chloroplast RFLP analysis (Zhang 1996) reports a higher level of variation within *Solidago*, and the resulting phylogeny strongly supports certain previously hypothesized groups. These contrasting levels of phylogenetic signal are best explained by the relative amount of DNA sequence scored in each study. Extrapolating from the values reported in Lane et al. (1996), approximately 2.5–3 kb of chloroplast sequence was assessed for variation in the Zhang study, compared to the approximately 1.1 kb ITS/ETS dataset analyzed here. In addition, previous work in Asteraceae indicates that chloroplast RFLP datasets can be more informative relative to those from ITS sequence (Morgan 1997). The most obvious possible source of the lack of ITS divergence in *Solidago* is that species level divergence is very recent. Other possible contributing factors include relatively large effective population sizes and the potential for homogenization through widespread interspecific hybridization followed by concerted evolution. Hybridization is traditionally viewed as widespread within *Solidago* (Cronquist 1980), and homogenization via concerted evolution is potentially a rapid process following hybridization (Franzke & Mummenhoff 1999). The relative lack of differentiation in ITS sequence between relatively distantly related congeners, which because of geographical considerations are unlikely to be part of a larger hybridization network, suggests that the primary cause is recency of divergence. This suggests that the striking ecological differences between *Solidago* species may have arisen during relatively short periods of time and indicates that resolving the phylogeny of *Solidago* will be a major challenge that will require markers evolving more rapidly than ITS and ETS.

APPENDIX

Collections of *Solidago* and related genera sampled for molecular analyses, with collector and herbarium or literature reference and GenBank numbers (ITS, ETS).

Solidago L. Solidaster cv. Lemore, *Schilling 07-06* (TENN), EU125353, EU125374. **Sect. Solidago, Subsect. Argutae,** *S. arguta* Aiton, *Beck 481* (MO), EU125354, EU125375; *S. patula* Muhl., *Beck 482* (MO), EU125355, EU125376. **Subsect. Glomeruliferae,** *S. caesia* L., *Beck 483* (MO), EU125356, EU125377; *S. flexicaulis L.*,Kress et al. 2005, DQ005979. **Subsect. Humiles,** *S. simplex* Kunth, Kress et al. 2005, DQ005982. **Subsect. Juncea,** *S. juncea* Aiton, Kress et al. 2005, DQ005981. **Subsect. Maritimae,** *S. sempervirens* L., Urbatsch et al. 2003, AF477668, AF477732. **Subsect. Nemorales,** *S. nemoralis* Aiton, *Estes 1521* (TENN), EU125357. **Subsect. Solidago,** *S. virgaurea* L., *Dinies s.n.* (MO), EU125358, EU125378; *S. decurrens* Lour., GenBank Record, EF103140. **Subsect. Squarrosae,** *S. speciosa* Nutt., *Schilling* 07-05 (TENN), EU125359. **Subsect. Thyrsiflorae,** *S. petiolaris* Aiton, Noyes & Rieseberg 1999, AF046968. **Subsect. Triplinerve,** *S. canadensis* L., Urbatsch et al. 2003, AF477665, AF477729; *Laferriere 3564* (TENN), EU125360, --; *Weldon I 7/22/81* (TENN), EU125361; *S. gigantea* Aiton, *Beck 509* (MO), EU125362, EU125379; *S. shortii* Torr. & A. Gray, Beck et al. 2004, AY523854, [submit]. **Subsect. Venosae,** *S. fistulosa* Mill., Urbatsch et al. 2003, AF477666, AF477730. **Sect. Ptarmacoidei** (*Oligoneuron*), *S. ptarmicoides* (Torrey & A. Gray) B. Boivin, *Brandt 2603* (MO), EU125363, EU125380; *Dietrich 524*

(MO), EU125364, EU125381; *Anderson MO221*, EU125365, EU125382; *S. nitida* Torrey & A. Gray, *Thomas 141724* (MO), EU125366, EU125383; *Thomas 97470* (MO), EU125367, EU125384; *Thomas 138143*, EU125368, EU125385; *S. ohiensis* Riddell, *Kral 48497* (MO), EU125369, EU125386; *S. riddellii* Frank, *Smith 3617* (MO), EU125370, EU125387; *Vogt* 506 (MO), EU125371, EU125388; *Luges s.n.* (MO), EU125372, EU125389; *S. rigida* L., Beck et al. 2004, AY523851, EU125390. **Brintonia** Greene, *B. discoidea* (Elliott) Greene, Roberts & Urbatsch 2003, AY170930, AY169727; *Anderson 20021* (MO), EU125373, EU125391. **Chrysoma** Nutt., *C. pauciflosculosa* (Michx.) Greene, Urbatsch et al. 2003, AF477637, AF477701. **Chrysothamnus** Nutt., *C. scopulorum* (M.E. Jones) Urbatsch, R.P. Roberts & Neubig, Roberts & Urbatsch 2003, AY170956, AY169753; *C. stylosus* (Eastwood) Urbatsch, R.P. Roberts & Neubig, Roberts & Urbatsch 2003, AY170973, AY169770.*C. viscidiflorus* Nutt., Roberts & Urbatsch 2003, AY170947, AY169744. **Columbiadoria** G.L. Nesom, *C. hallii* (A. Gray) G.L. Nesom, Roberts & Urbatsch 2003, AY170948, AY169745. **Cuniculotinus** Urbatsch, R.P. Roberts & Neubig, *C. gramineus* (H. M. Hall) Urbatsch, R.P. Roberts & Neubig, Roberts & Urbatsch 2003, AY170936, AY169733. **Eastwoodia** Brandegee, *E. elegans* Brandegee, Roberts & Urbatsch 2003, AY170949, AY169746. **Lorandersonia** Urbatsch, R.P. Roberts & Neubig, *L. linifolia* (Greene) Urbatsch, R.P. Roberts & Neubig, Roberts & Urbatsch 2003, AY170936, AY169737. **Oreochrysum** Rydb., *O. parryi* Rydb., Roberts & Urbatsch 2003, AY170958, AY169755. **Petradoria** Greene, *P. pumila* Greene, Roberts & Urbatsch 2003, AY170959, AY169756. **Sericocarpus** Nees, *S. tortifolius* Nees, Urbatsch et al. 2003, AF477664, AF477728. **Stenotus** Nutt., *S. acaulis* Nutt., Roberts & Urbatsch 2003, AY170960, AY169757. **Tonestus** A. Nelson, *T. pygmaeus* A. Nelson, Roberts & Urbatsch 2003, AY170972, AY169769.

ACKNOWLEDGMENTS

The authors thank G. Beattie, P. Heise, and J. Miller for technical support, and G. Nesom and J. Semple for helpful comments on the manuscript. Financial support from the L.R. Hesler Fund of the Department of Botany, University of Tennessee, and NIH award #P20 RR164841, is gratefully acknowledged.

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